REMARKS

Claims 7-12, 15-17, 22-25 and 29-37 are currently pending in this application, where claims 22-25 have been withdrawn as being directed to non-elected subject matter. Claims 1-6, 13-14, 18-21, 26-28 and 38 were previously cancelled.

Applicants thank the Examiner for the withdrawal of previous two objections to the specification and to claim 12 and for the withdrawal of a previous obviousness rejection over Zhang et al. in view of Venugopal et al. Applicants also thank the Examiner for the indication that claims 30 and 34 are allowable and claims 12, 15, 33 and 37 will be allowable, if rewritten in independent claim form.

A listing of claims is presented to show claim 10 without mark-up, which was inadvertently retained from a prior amendment in the last response to the Official Action.

The Specification has been amended in this paper to include a statement as required by 37 CFR §1.808 with regard to the deposit requirement for microorganisms.

No new matter has been introduced in the claims and the specification within the meaning of 35 U.S.C. §132. Accordingly, entry of the amendments to the claims and the specification is respectfully requested.

In view of the amendment and the following remarks, Applicants respectfully submit that the pending claims are in condition for allowance.

I. Objection to Claim 10

Applicants respectfully submit that currently pending claim 10 has been corrected to remove the amendment brackets and the underlined word which was made in the prior amendment filed on March 14, 2008. Accordingly, withdrawal of this objection is respectfully requested.

II. Rejection of Claims 16, 17, 32 and 36 under 35 USC §112, 1st Paragraph

Applicants respectfully submit that the Specification has been amended to include the statement, "All restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of a patent," as required by 37 CFR §1.808. Accordingly, withdrawal of this rejection is respectfully requested.

III. Rejection of Claims 7-11, 29, 31 and 35 under 35 USC §112, 1st Paragraph

The Examiner rejects claims 7-11, 29, 31 and 35 under 35 USC §112, first paragraph, as failing to comply with the written description requirement. As the basis for this rejection, the Examiner asserts in relevant part of the Official Action:

> A review of the language of the claims indicates that these claims are drawn to a genus, i.e., a BAC vector encoding a full-length cDNA of JEV, wherein the cDNA clone comprises a promoter. Claims 31 and 35 are drawn to a genus, i.e., pBAC^{SP6}/JVFLxl*Xba*l and pBAC^{T7}/JVFLxl*Xba*l. … There is a single species of the claimed genus disclosed that is within the scope of the claimed genus, i.e., SEQ ID NO:45 and SEQ ID NO:48. The disclosure of a single disclosed species may provide an adequate written description of a genus when the species disclosed is representative of the genus. However, the present claim encompasses

> numerous species that are not further described. There is substantial variability among the species. In the absence of sufficient recitation of distinguishing characteristics, the specification does not provide adequate written description of the claimed genus, which is pBAC^{SP6}/JVFLxl*Xba*I and pBAC^{T7}/JVFLxl*Xba*I or a BAC vector encoding a full-length cDNA of JEV, wherein the cDNA clone comprises a promoter. … Amending claims 31 and 35 to include the SEQ ID NOs should obviate part of the rejection. In the alternative, amending the claims so that claim 31 is dependent on claim 30 and claim 35 is dependent on claim 34 should obviate part of the rejection.

Applicants respectfully traverse this rejection. Applicants submit that currently pending claims 7-11, 29, 31 and 35 fully comply with the written description requirement of 35 USC § 112, first paragraph, as set forth in the below.

The test under 35 U.S.C. 112, first paragraph, for determining compliance with the written description requirement is whether the application clearly conveys that an applicant has invented the subject matter which is claimed. *In re Barker*, 194 USPQ 470, 473 (CCPA 1977); MPEP 2163. The applicant must convey to the public what the applicant claims as the invention so that the public may ascertain if the patent applicant claims anything in common use or already known. MPEP § 2163. Lastly, the specification must convey that the applicant was in possession of the invention. MPEP § 2163. In this regard, the Examiner is respectfully reminded that the Examiner has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. *In re Wertheim*, 191USPQ 90, 98 (CCPA 1976).

Presently Claimed Subject Matter

Pending independent claim 7 recites "A full-length infectious and genetically stable cDNA clone of Japanese encephalitis virus (JEV), wherein a full-length cDNA of JEV is cloned into a bacterial artificial chromosome (BAC) and an infectious RNA transcript of JEV is transcribed directly from the cDNA clone." Pending independent claim 31 recites "A vector, comprising: a full-length infectious and genetically stable cDNA clone of Japanese encephalitis virus (JEV), wherein the vector is pBAC^{SP6}/JVFLx/*Xba*I." Independent claim 35 recites "A vector, comprising: a full-length infectious and genetically stable cDNA clone of Japanese encephalitis virus (JEV), wherein the vector is pBAC^{T7}/JVFLx/*Xba*I."

Accordingly, it is clear that the presently claimed subject matter is ***not*** a DNA itself, but a full-length infectious and genetically stable cDNA clone of JEV which is cloned into a BAC, in other words, ***recombinant BAC plasmid comprising a full-length infectious and genetically stable cDNA of JEV***. The technical feature of the present subject matter is in use of a BAC plasmid for cloning a full-length cDNA of JEV. The cloning of a full-length cDNA of JEV into the BAC plasmid makes it possible to construct genetically stable full-length cDNA clones of JEV, as well as to produce an infectious full-length RNA transcript directly from the clone. This subject matter was accomplished by Applicants for the first time.

In this regard, the present specification provides sufficient disclosure and working examples supporting the full scope of claims. By the detailed descriptions

and the working examples, the specification clearly conveys that Applicants were in possession of the claimed subject matter at the time the present application was filed.

In particular, Applicants constructed various recombinant BAC plasmids containing some full-length cDNAs of JEV, including pBAC ^{SP6}/JVFL/ Xho I, pBAC ^{T7} /JVFL/ Xho I, pBAC^{SP6}/JVFLx/Xho I, pBAC ^{T7}/JVFLx/ Xho I, pBAC ^{SP6}/JVFLx/ Xba I, pBAC ^{T7} /JVFLx/ Xba I, pBAC ^{SP6} /JVFLx/ Xba I ^{MBN}, pBAC ^{T7} /JVFLx/ Xba I ^{MBN}, pBAC ^{SP6} /JVFLx/ GFP/ Xba I, pBAC ^{SP6} /JVFLx/ LUC/ Xba I, pBAC ^{SP6} /JVFLx/ GFP/ Xba I ^{MBN}, pBAC ^{SP6} /JVFLx/ LUC/ Xba I ^{MBN}, pJEV/FL/LUC, pJEV/FL/LacZ, pJEV/FL/EGFP, pJEV/FL/PAC, etc. *See* Table 3, Examples 9 and 10. Some constructs among them contain heterologous genes which are not derived from JEV genome. Thus, the recombinant BAC plasmid containing a full-length cDNA of JEV is independent from the nucleotide sequences of the cDNA.

Also, all of the BAC constructs are capable of infecting cells and producing infectious JEV particles after infection. *See* Table 3 and the fifth paragraph of Example 9. Accordingly, it is very clear from the description in the specification that Applicants were in possession of the claimed subject matter, i.e., a recombinant BAC plasmid comprising a full-length infectious and genetically stable cDNA of JEV, at the time the present application was filed.

In this regard, it should be noted that all BAC vectors share a number of structural components, for example, *oriS* and *repE-F* which are involved in plasmid replication, and copy number regulation, *parA* and *parB* which are involved in F-plasmid partitioning during cell division and the stable maintenance of the BAC, and

T7 and/or SP6 promotor(s) which is responsible for the transcription of inserted genes. As a result, all BAC vectors share a number of functional features, for example, the property of being evenly distributed and stably maintained at low copy number following cell divisions, and the capacity to accommodate large inserts. In the present application pBeloBAC11 were used, which is in fact a derivative of pBAC108L, which most commercially available BAC vectors are based on, being different from the latter only by the presence of an LacZ. Due to the high degree of structural and functional similarities, a person skilled in the art would have been readily recognized that stable full-length JEV cDNA clones can be obtained using BAC vectors other than pBeloBAC11.

Also, it should be noted that unstable JEV cDNA clones have a high propensity for rearrangements. *See* Yamshchikov et al. (Virology, 281(2): 272-280, 2001) at page 273, left col., last paragraph, lines 3-5. A copy of the reference was submitted in the IDS filed on January 21, 2009. cDNA clones of the present subject matter had stable restriction endonuclease digestion pattern after repeated passage, which is a good indicator that the cDNA clones were stable. It was also known in the art that nonsense mutations are often times found in the E gene of JEV cDNA clone. *See* Yamshchikov et al. page 273, left col., last paragraph, lines 5-8. These nonsense mutations would result in the production of truncated E gene products with impaired function, which would compromise the infectivity of the JEV RNA. The synthetic RNAs transcribed from the cDNA clones of the present subject matter had high infectivity, which is yet another good indicator that the cDNA clones were stable.

Accordingly, even with no nucleotide sequences, it is very clear from the specification that the present application provided a genetically stable full-length JEV cDNA clones.

In view of the foregoing, Applicants respectfully submit that the present specification fully meets the written description requirement with regard to claims 7-11, 29, 31 and 35, as required by 35 U.S.C. §112, first paragraph. Therefore, reconsideration and withdrawal of this rejection is respectfully requested.

IV. Rejection of Claims 7-11, 29, 31 and 35 under 35 USC §103(a)

The Examiner rejects claims 7-11, 29, 31 and 35 as being unpatentable over Zhang et al. (Journal of Virological Methods, Aug.2001, Vol. 96, No. 2, pp. 171-182) in view of Almazán et al. (PNAS, May 2000, Vol. 97, No. 10, pp 5516-5521).

As a basis for the rejection, the Official Action states in relevant part:

> Zhang et al. also teach that the transcript from the clone was non-infectious, however, the transcript from the amplicon of the clone was infectious. … However, Zhang does not teach the cDNA of JEV cloned into a BAC vector and is silent to whether the JEV cDNA clone is infectious.
>
> Almazán teaches cDNA clones of single-stranded, positive-sense RNA viruses (JEV is a single-stranded RNA virus) which were cloned into a bacterial artificial chromosome (BAC) vector (p. 5516, Abstract and 1st paragraph). Almazán teaches removal of restriction endonuclease in the TGEV sequence generated a stable plasmid (p. 5518, 2nd col., 2nd full para.) Almazán teaches BACs have been useful to clone large DNAs stably from a variety of complex genomic source (p. 5516, 3rd para.). However, Almazán does not teach a full-length cDNA of JEV. Since claims 31 and 35 are drawn to a generic BAC vector comprising a JEV cDNA and a promoter known in the art, Zhang and Almazán teach the limitations of these claims.

> It would have been *prima facie* obvious to the person of ordinary skill in the art at the time the invention was made to clone the cDNA of JEV taught by Zhang into a BAC vector because Almazán teaches BACs have been useful to clone large DNAs stably with a reasonable expectation of success.

Applicants respectfully traverse this rejection. The cited references, together, do not establish a *prima facie* case of obviousness against presently pending claims 7-11, 29, 31 and 35.

To establish a *prima facie* case of obviousness, the PTO must satisfy three requirements. First, as the U.S. Supreme Court recently held in *KSR International Co. v. Teleflex Inc., 550 U.S. 398 (2007)*, "a court must ask whether the improvement is more than the predictable use of prior art elements according to their established functions. ... it can be important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does... because inventions in most, if not all, instances rely upon building blocks long since uncovered, and claimed discoveries almost of necessity will be combinations of what, in some sense, is already known." Second, the proposed modification of the prior art must have had a reasonable expectation of success, determined from the vantage point of the skilled artisan at the time the invention was made. *Amgen Inc.* v. *Chugai Pharm. Co.*, 18 USPQ 1016, 1023 (C.C.P.A 1970). Lastly, the prior art references must teach or suggest all the limitations of the claims. *In re Wilson*, 165 USPQ 494, 496 (C.C.P.A. 1970).

In the present application, Applicants submit that a *prima facie* case of obviousness has not been established by the Examiner since there is no apparent

reason to combine the prior art elements in the way claimed, as well as there is no reasonable expectation of success to reach the presently claimed subject matter.

Presently claimed subject matter

As clearly presented in broadest claim 7, the present subject matter is drawn to "A full-length infectious and genetically stable cDNA clone of Japanese encephalitis virus (JEV), wherein ***a full-length cDNA of JEV is cloned into a bacterial artificial chromosome (BAC)*** and an infectious RNA transcript of JEV is transcribed ***directly*** from the cDNA clone." All of claims 8-11, 21 and 29 directly or indirectly, dependent from claim 7 and thus contain all the limitations of claim 7 as above. Claims 31 and 35 are directed to a vector, comprising: a full-length infectious and genetically stable cDNA clone of Japanese encephalitis virus (JEV), wherein the vector is $pBAC^{SP6}$/JVFLx/*Xba*I (claim 31) or $pBAC^{T7}$/JVFLx/*Xba*I (claim 35).

Zhang et al.

As the Examiner expressly concedes in the Official Action, Zhang et al. fail to teach or suggest "infectious" cDNA clone of JEV, as well as the use of bacterial artificial chromosome (BAC) vector.

In this regard, the discussion of Zhang et al. presented in the previous response is incorporated herein. To summarize, Zhang et al. describe a genetically stable cDNA clone of JEV, but that is non-infectious. Zhang et al. describe only an amplicon (a cDNA molecule, not a clone) as infectious, which is produced by long

RT-PCR using the cDNA clone as a template and RNA transcripts transcribed from the amplicon. An infectious cDNA "clone" is distinguished from an infectious cDNA "molecule" since the former is capable of self-replicating, but the latter isn't. Actually, an infectious cDNA molecule of JEV which is not cloned is well known in the art. However, prior to the present application, no one had succeeded in producing an infectious cDNA clone of JEV, including Zhang et al. It has been recognized in the art that an infectious cDNA clone of a particular RNA virus is a "cloned cDNA capable of producing infectious RNA transcripts transcribed therefrom." Nowhere do Zhang et al. describe that they produced an infectious cDNA clone of JEV. Rather, Zhang et al. describe that they constructed a stable genome-size cDNA clone of JEV. Accordingly, Zhang et al. recognize the difference between an infectious cDNA clone of JEV and a simple full-length cDNA clone of JEV. In addition, Zhang et al. do not teach or suggest at all the use of a BAC vector, to produce an infectious full-length cDNA clone of JEV.

Accordingly, it is very clear that Zhang et al., taken alone, do not teach or suggest the present subject matter. Furthermore, Almazán et al. do not remedy the deficiencies of Zhang et al.

Almazán et al.

Almazán et al. disclose engineering the largest RNA virus genome, i.e., ***coronavirus*** (TGEV) genomes, as an infectious bacterial artificial chromosome

(BAC). However, ***nowhere*** do Almazán et al. disclose JEV or flavivirus, but instead disclose TGEV which is a coronavirus.

In this regard, the Examiner indicates that JEV is a single-stranded, positive sense RNA virus, like coronavirus. However, although both viruses are single-stranded, positive-sense RNA viruses, the similarity stops short with these common features. The specific technical problem of JEV cDNA stability cannot be studied or solved by working with coronaviruses. For example, the genome size of TGEV is about 30 kb which is largest genomes among RNA viruses. In contrast, JEV has only 11 kb genome size.

In addition, Almazán *et al*. describe that "the enormous length of the coronavirus genome and the instability of plasmids carrying coronavirus replicase sequences have thus far hampered the construction of a full-length cDNA clone." To overcome the problems, Almazán et al. teach, a stable full-length coronavirus cDNA clone was obtained using a ***combination of three strategies***: (i) constructing the full-length cDNA from a defective minigenome which was devoid of the toxic fragments and reintroducing the most toxic fragment in the last cloning step; (ii) cloning the cDNA in a BAC vector; and (iii) producing RNA in the nucleus from the CMV promoter. *See*, page 5516, from left col., last paragraph to right col., first paragraph. With regard to strategy (ii), "cloning the cDNA as BAC," Almazán et al. describe that BACs have been useful to clone ***large DNAs*** stably from a variety of complex genomic sources into bacteria, including herpesvirus DNA. That is, Almazán et al. used a BAC for cloning of cDNA of coronavirus because BACs have

been useful to clone "large DNAs." Accordingly, Almazán et al. teach that using a BAC vector can partially solve the problem of instability caused by a very "large viral genome."

Thus, Applicants submit that there is ***no apparent reason*** to motivate a person skilled in the art to combine the teachings of Almazán et al. with Zhang et al. to reach the presently claimed subject matter, as required by *KSR International Co. v. Teleflex Inc.* "The Examiner must identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does," (KSR Opinion at p. 15).

More specifically, coronavirus has a genome of 30 kb which is very large. In contrast, JEV has a genome of only 11 kb, which is not considered very large by a person skilled in the art. Further, there is no indication in the prior art that the instability of a full-length JEV cDNA clone is caused by the size of its genome. While for TGEV the stability problem is believed to be due to the large size of the genome, the problem with respect to the stability of the JEV genome is due to specific sequences which are prone to rearrangements and mutations. *See*, e.g., Yamshchikov et al., page 273, right col. Thus, in order to solve the problem of the large size of the TGEV genome, the use of vectors with a large capacity, like BACs as described in Almazán et al., might be regarded as obvious. However, due to the different technological problem with respect to the JEV genome, a person skilled in the art would have had no reason to combine the teachings of Zhang et al. with the

teachings of Almazán et al., namely the use of a BAC vector, to stabilize the JEV genome.

Furthermore, Almazán et al. teach that it was the combination of three strategies that enabled the construction of a stable full-length coronavirus cDNA clone. Accordingly, a person skilled in the art would have rather been taught that cloning a full-length JEV cDNA into a BAC vector "alone" is not sufficient to achieve a stable full-length JEV cDNA clone, which, in contrast, was achieved by the present application.

The above assertion is further supported by the disclosure in Shizuya *et al.*, (*Proc. Natl. Acad. Soc. U.S.A.*, 89: 8794-8797, 1992) below, which reference is cited in Almazán et al. and a copy of which is submitted herewith in an IDS form.

> The BAC system is based on E. Coli and its ingle-copy plasmid F factor. It is capable of maintaining human genomic DNA fragments of >300 kb pairs. Individual clones of human DNA appear to be maintained with a high degree of structural stability in the host, even after 100 generations of serial growth. *See* Abstract.

Thus, a person skilled in the art would have thought that use of a BAC vector is just for cloning large DNA stably. Indeed, the size of JEV genome is only 11 kb, one third of that of coronavirus. Accordingly, a person skilled in the art would have rather tried to use a phagemid or a cosmid for cloning cDNA of JEV. However, an attempt to clone an infectious cDNA of JEV into a cosmid was a complete failure. *See* Zhang *et al*.

In addition, for many RNA viruses, the genetic instability of the cloned cDNA during its construction is particularly problematic. JEV is a RNA viruse. Thus, in spite

of previously conducted extensive attempts, it has been reported that obtaining a genetically stable full-length infectious JEV cDNA clone was very difficult. *See* page 20, line 7 to 20 of the specification. For example, Yamshchikov *et al*. teach difficulties for cloning a full-length infectious cDNA of JEV, even citing Almazán *et al.*:

> Japanese encephalitis (JE) virus belongs to the Flaviviridae family of (+)RNA virus, which also includes yellow fever (YF), West Nile, tick borne encephalitis, dngue, and hepatitis C virus. Flaviviruses, in particular JE (Sumiyoshi et al., 1992) and YF (Rice et al., 1989), represent the most striking example of highly unstable infectious clones, which have been composed by in vitro ligation. [Underline added] *See* page 273, left col. lines 16- 23.
>
> Accordingly, the stabilizing mutation now has been found upstream form the prM signal sequence, and insertion of a second copy of the intron upstream from this region has resulted in complete stabilization of the construct. Removal of the intron at i2217 again destabilized the plasmid, suggesting initiation of spurious transcription from an upstream element. The infectious clone for YF virus also was unstable under control of the bacteriophage promoter (Rice et al., 1989), suggesting the existence of an internal promoter-like sequence(s) in the YF genome. For a recently reported coronavirus infection clone (Almazan *et al*., 2000), destabilizing sequences found inside the virus genome might also indicate the existence of such element. It would be interesting to test whether YF and coronavirus infectious clones can be substantially stabilized by inserting temporary translation blocks upstream from the problematic genome regions. See page 277, right col. lines 2-18.

Accordingly, it was known in the art that JEV is one of the most striking example of highly unstable infectious clone, and the instability may be due to spurious transcription initiated from a prokaryotic promoter-like sequence. In Yamshchikov et al., the problem was overcome by introducing two artificial intron into the problematic genome region. Further, Yamshchikov *et al*., suggest that instability of coronavirus may be due to destabilizing sequences found inside the virus genome. Yamshchikov *et al*. suggest that technical solution to achieve infectious clone of coronavirus is removing of

the destabilizing sequence rather than inserting cDNA of coronavirus into a BAC plasmid.

In view of the foregoing, there is no reason why a person skilled in the art would combine the cited references to reach the present subject matter.

Further, Applicants note that cDNA clone of Almazán et al. is ***not*** genetically stable since an RNA molecule rescued from a cell infected with a BAC containing TGEV cDNA exhibited 5 mutations. *See* page 5519, right col. last paragraph. Applicants also note that the system of Almazán et al. is "DNA-launched," i.e. it involves the translocation of the cDNA into the nucleus and a subsequent translocation of transcribed pre mRNA into the cytoplasm. Both processes are, however, not compatible with JEV since the infectiousness of JEV depends on the exact sequences of the 5'-end and the 3'-end of the JEV RNA molecule. Any additional nucleotides at the 5'-or the 3' end of the JEV RNA lower the infectivity of JEV. *See*, e.g., page 21, line 11 to page 23, line 12 of the present specification. Almazán et al. do not contemplate the removal of additional nucleotides at both ends, instead the addition of a synthetic poly(A) tail followed by further sequences is taught. *See* page 5517, last paragraph of Construction of the TGEV Full-Length cDNA section. Thus, the modifications of the TGEV genome presented in Almazán et al. do not apply to the JEV genome.

Accordingly, Applicants submit that there is no reasonable expectation of success to reach the present subject matter by the combination of both the references.

In view of foregoing, there is no apparent reason for a person skilled in the art to be promoted to combine the references to reach the present subject matter.

Further, if combined by any reason, there is no reasonable expectation of success to reach the present subject matter.

Accordingly, nothing in Zhang et al. and Almazán et al., each of reference being taken alone or in combination, can render the presently claimed subject matter obvious under the meaning of 35 USC 103(a).

Lastly, Applicants direct the Examiner's attention to the fact that corresponding European and Japanese patent applications have been granted to a patent through the examination by the EPO (EP1556493 B1) and the JPO (JP 4430543 B1), respectively, with the same scope of claims as the presently pending claims. Especially, the European Application was allowed overcoming the rejection by the Examination Division of the EPO for lacking inventive step over Almazán *et al.* and Yamshchikov *et al.*

Therefore, Applicants respectfully request the Examiner to reconsider and withdraw this rejection.

CONCLUSION

In view of the foregoing, Applicants submit that the pending claims are in condition for allowance. Early notice to this effect is earnestly solicited. The Examiner is invited to contact the undersigned attorney if it is believed such contact will expedite the prosecution of the application.

If the Examiner has any questions or comments regarding this matter, he is welcomed to contact the undersigned attorney at the below-listed number and address.

In the event this paper is not timely filed, applicants petition for an appropriate extension of time. Please charge any fee deficiency or credit any overpayment to Deposit Account No. 14-0112.

Respectfully submitted,

THE NATH LAW GROUP

Date: May 14, 2010

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